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CARBONIC ANHYDRASE INHIBITORS: AROMATIC SULFONAMIDES AND DISULFONAMIDES ACT AS EFFICIENT TUMOR GROWTH INHIBITORS[#]

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Aromatic/heterocyclic sulfonamides generally act as strong inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). Here we report the unexpected finding that potent aromatic sulfonamide inhibitors of CA, possessing inhibition constants in the range of 10^{-8}–10^{-9} M (against all the isozymes), also act as efficient *in vitro* tumor cell growth inhibitors, with GI_{50} (molarity of inhibitor producing a 50% inhibition of tumor cell growth) values of 10 nM–35 μM against several leukemia, non-small cell lung cancer, ovarian, melanoma, colon, CNS, renal, prostate and breast cancer cell lines. The investigated compounds were sulfanilyl-sulfanilamide-, 4-thioureido-benzenesulfonamide- and benzene-1,3-disulfonamide-derivatives. The mechanism of antitumor action with these sulfonamides is unknown, but it might involve either inhibition of several CA isozymes (such as CA IX, CA XII, CA XIV) predominantly present in tumor cells, a reduced provision of bicarbonate for the nucleotide synthesis (mediated by carbamoyl phosphate synthetase II), the acidification of the intracellular milieu as a consequence of CA inhibition or uncoupling of mitochondria and potent CA V inhibition among others. A combination of several such mechanisms is also plausible. Optimization of such derivatives from the SAR point of view, might lead to the development of effective novel types of anticancer agents/therapies.

INTRODUCTION

A large number of different carbonic anhydrase (CA, EC 4.2.1.1) isozymes have been described up to now in higher vertebrates, including humans.

[#] See ref. [1]

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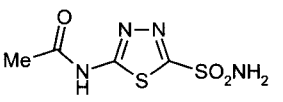
These enzymes are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH homeostasis, electrolyte secretion in a variety of tissues/organs, or biosynthetic reactions, such as the lipogenesis, gluconeogenesis and ureagenesis among others.[2–6] From the sub-cellular location point of view, some of these isozymes are present in the cytosol (CA I, CA II, CA III, CA VII), others are membrane-bound (CA IV, CA IX, CA XII and CA XIV), CA V is present only in mitochondria, CA VI is secreted in saliva, whereas several acatalytic forms are also known (CA VIII, CA X and CA XI).[2–14]



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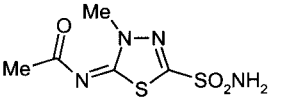

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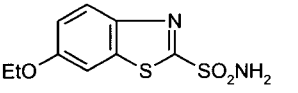

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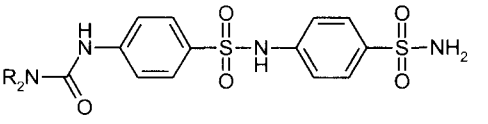
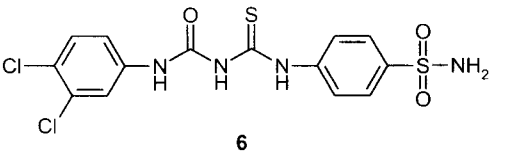
Recently, three novel membrane-bound CA isozymes, CA IX,[8] XII[11] and XIV,[12] in addition to the "classical" one, CA IV,[13] have been isolated and characterized. Some of them were identified predominantly in tumor cells, and little is known for the moment regarding the physiological consequences of their inhibition/activation.[11–14] Although inhibition of CAs by aromatic/heterocyclic sulfonamides has been clinically exploited for more than 45 years in the treatment of a variety of diseases such as glaucoma,[4,15] epilepsy,[16] congestive heart failure,[4] mountain sickness,[17] gastric and duodenal ulcers,[18] or as diuretic agents,[19] their potential use as antitumor drugs has little been explored up to now. Several classical clinical agents from this class include acetazolamide **1**, methazolamide **2** or ethoxzolamide **3**.[4] In a seminal work, Teicher *et al.*[20] reported that one of these derivatives, acetazolamide **1**, which behaves as a strong inhibitor of several CA isozymes (CA II, CA IV, CA V and CA VII among others),[3–5] is a potential modulator of anticancer therapies in combination with different cytotoxic agents (alkylating agents, nucleoside analogs, platinum derivatives, etc), probably due to the acidification of the intratumoral environment ensued after CA inhibition, although other mechanisms of action of this drug were not excluded. Our and Puscas's groups[21a] also showed earlier that by modulating CA activity (by means of inhibitors or activators of this enzyme) the pH of the tumor environment can be changed, which may favorably influence the anticancer effect of the drug *per se* (i.e., the sulfonamide CA inhibitor) or that of another anticancer agent used concomitantly with the CA inhibitor/activ-

ator. Chegwidden and Spencer[21b] then showed that potent, clinically used sulfonamide CA inhibitors, such as methazolamide **2**, or ethoxzolamide **3** (in concentrations of 0.4–1 mM) strongly inhibited *in vitro*, in cell cultures, the growth of human lymphoma cells showing that this is mainly due to a reduced provision of bicarbonate for nucleotide synthesis (HCO_3^- is the substrate of carbamoyl phosphate synthetase II) as a consequence of CA inhibition.[21b] As mentioned above, some new CA isozymes, such as CA IX,[8] CA XII[11,14] and CA XIV[12] are highly abundant in tumor cells. These and other more classical isozymes, such as CA I, II and IV,[22,23] were also recently shown to be present and actively involved in other types of proliferative conditions, such as von Hippel-Lindau tumors,[14] progressive polycystic kidney disease,[22] acinar-ductal carcinomas of the pancreas,[23a] autoimmune or idiopathic chronic pancreatitis,[23b] as well as apoptotic processes in some human pancreatic cancer cells.[23c] It appeared thus of interest to further explore the connections between CAs and tumors, and the development of specific inhibitors for some of the isozymes presumably involved in such processes would be highly beneficial for both obtaining novel types of drugs as well as for a better understanding of the physiology of the CAs.



4: R = H; **5**: R = Ph



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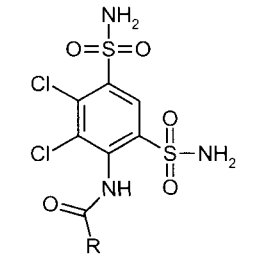
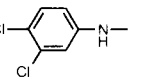


7: R = 

8: R = Ph_2N-

In previous contributions from this laboratory[15,24–27] it was shown that by attaching different "tails" to the molecules of aromatic/heterocyclic sulfonamides, it was possible to obtain potent CA inhibitors possessing the desired physico-chemical properties, such as enhanced water solubility (for their use as antiglaucoma drugs),[15,24–26] membrane impermeability (important for obtaining isozyme-specific inhibitors),[27] etc., all requisites of interest for the design of novel types of pharmacological agents. Here we

report the unexpected finding that several aromatic sulfonamide/disulfonamides incorporating such a di-/trisubstituted-urea/thiourea "tail", of types **4–8**, and possessing strong CA inhibitory properties, also proved to be effective *in vitro* tumor cell growth inhibitors of different leukemia, non-small cell lung cancer, melanoma, ovarian, renal, prostate and breast cancer cell lines. As far as we know, this is one of the first systematic studies reporting sulfonamide CA inhibitors with such high potency as *in vitro* tumor cell growth inhibitors, against such a variety of tumor cell types/lines.

MATERIALS AND METHODS

Melting points were done on a heating plate microscope (not corrected). IR spectra were obtained with KBr pellets, 400–4000 cm^{-1} using a Perkin-Elmer 16PC FTIR spectrometer and ^{1}H-NMR spectra with a Varian 300CXP (chemical shifts are expressed as δ values relative to Me_4Si as standard). Elemental analysis was done with a Carlo Erba Instrument CHNS Elemental Analyzer, Model 1106. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm precoated silica gel plates (E. Merck). Sulfonamides **4–8** were prepared as described previously: the sulfanilyl-sulfanilamide **4** as described in reference[28]; compounds **5** and **8** are novel and are described here (see later in the text); **6** and **7** are described in reference.[29] Sulfonamides used in the synthesis, N,N-diphenylcarbamoyl chloride, solvent and other reagents used were from E. Merck, Fluka, or Sigma-Aldrich and were used without further purification. Solvents used in these experiments were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

4-[4-(N,N-Diphenylcarbamoylamido)benzenesulfonylamido]-benzenesulfonamide **5**. An amount of 320 mg (1 mMole) of sulfanylyl-sulfanilamide[30] was suspended in 50 mL of dry acetonitrile and the stoichiometric amount of N,N-diphenyl carbamoyl chloride (232 mg) and triethylamine (108 μL), dissolved in the same solvent, were added to the reaction mixture which was stirred at 4°C for 4–5 hours (TLC control). The solvent was evaporated *in vacuo*, the residue taken up in 50 mL of cold water, brought to pH 5 with 5% citric acid and the precipitated sulfonamide filtered and recrystallized from ethanol (87% yield). White crystals, m.p. 278–9°C, IR (KBr), cm^{-1}: 1176 (SO_2), 1365 (SO_2), 1540 (C=O, amide II), 1670 (C=O, amide I), 3060 (NH, NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.78 (d, 2H, ArH *ortho* to the SO_2NH_2 group), 6.84 (2H, ArH *ortho* to the SO_2NH group), 7.41 (d, 2H, ArH meta to SO_2NH_2group), 7.45 (d, 2H, ArH meta to SO_2NH group),

7.08–7.79 (m, 10H, 2 Ph), 7.95 (s, 1H, CONH), 8.21 (br s, 2H, SO_2NH_2), 8.64 (br s, 1H, SO_2NH). Found: C, 57.29; H, 4.36; N, 10.65. $C_{25}H_{22}N_4O_5S_2$ requires: C, 57.46; H, 4.24; N, 10.72%.

4-(N,N-Diphenylcarbamoylamido)-5,6-dichloro-1,3-benzenedisulfonamide **8**. An amount of 320 mg (1 mMole) of 4-amino-5,6-dichlorobenzene-1,3-disulfonamide was suspended in 50 mL of dry acetonitrile and the stoichiometric amount of N,N-diphenyl carbamoyl chloride (232 mg) and triethylamine (108 μL), dissolved in the same solvent, were added to the reaction mixture which was stirred at 4°C for 4–5 hours (TLC control). The solvent was evaporated *in vacuo*, the residue taken up in 50 mL of cold water, brought to pH 5 with 5% citric acid and the precipitated sulfonamide filtered and recrystallized from ethanol-water 1:1, v/v (78% yield). White crystals, m.p. > 300°C, IR (KBr), cm^{-1}: 1160 (SO_2), 1378 (SO_2), 1540 (C=O, amide II), 1670 (C=O, amide I), 3060 (NH, NH_2); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.08–7.71 (m, 10H, 2 Ph), 7.76 (s, 1H, ArH from the substituted benzene-disulfonamide moiety), 7.89 (s, 1H, CONH), 8.24 (br s, 4H, 2 SO_2NH_2). Found: C, 44.52; H, 3.30; N, 10.71. $C_{19}H_{16}Cl_2N_4O_5S_2$ requires: C, 44.28; H, 3.13; N, 10.87%.

Enzyme Preparations

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Lindskog *et al.*[31] (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by this group,[32] and enzymes were purified by affinity chromatography according to the method of Khalifah *et al.*[33] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 $mM^{-1} \cdot cm^{-1}$ for CA I and 54 $mM^{-1} \cdot cm^{-1}$ for CA II, respectively, based on $M_r = 28.85$ kDa for CA I and 29.30 kDa for CA II, respectively.[34,35] CA IV was isolated from bovine lung microsomes as described by Maren *et al.*, and its concentration was determined by titration with ethoxzolamide.[36]

Initial rates of 4-nitrophenyl acetate hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nM, with a Cary 3 instrument interfaced with an IBM compatible PC.[37] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between $2 \cdot 10^{-2}$ and $1 \cdot 10^{-6}$ M, working at 25°C. A molar absorption coefficient ε of 18,400 $M^{-1} \cdot cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis under the conditions of the experiments (pH 7.40), as reported

in the literature.[37] Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Duplicate experiments were done for each inhibitor concentration and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in DMSO and dilutions up to 0.01 nM were done thereafter with distilled-deionized water (DMSO is not inhibitory at these concentrations). Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay in order to allow for the formation of the E-I complex. The inhibition constant K_I was determined as described in reference.[37] Enzyme concentrations were 3.4 nM for hCA II, 10.3 nM for hCA I and 24 nM for bCA IV (this isozyme has a decreased esterase activity[13] and higher concentrations had to be used for the measurements).

Inhibition of Tumor Cell Growth

Stock solutions of inhibitor (1 mM) were prepared in DMSO and dilutions up to 10 nM done with distilled deionized water. The percentual growth (PG) of the cell lines in the presence of five concentrations (10^{-8}–10^{-4} M) of inhibitor was calculated according to one of the following two expressions (1) or (2):[38]

$$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_0)/(\text{Mean } OD_{ctrl} - \text{Mean } OD_0),$$
$$\text{when } (\text{Mean } OD_{test} - \text{Mean } OD_0) \geq 0, \quad (1)$$

$$PG = 100 \times (\text{Mean } OD_{test} - \text{Mean } OD_0)/(\text{Mean } OD_0),$$
$$\text{when } (\text{Mean } OD_{test} - \text{Mean } OD_0) < 0, \quad (2)$$

where Mean OD_0 = the average optical density measurements of sulforhodamine B (SRB)-derived color just before exposure of cells to the test compounds; Mean OD_{test} = the average optical density measurements of SRB-derived color after 48 h exposure of cells to the test compounds; Mean OD_{ctrl} = the average optical density measurements of SRB-derived color after 48 h with no exposure of cells to the test compounds. GI_{50} represents the molarity of inhibitor producing a 50% inhibition of growth of the tumor cells after 48 h exposure to variable concentrations (10^{-4}–10^{-8} M) of the test compound, measured as outlined before, and this parameter was obtained by interpolation. GI_{50} is in fact the molarity of inhibitor at which PG = 50%.[38] The standard suforhodamine B (SRB) protein assay has been used to estimate cell viability or growth.[38]

RESULTS AND DISCUSSION

Although a very large number of aromatic/heterocyclic sulfonamide CA inhibitors have been synthesized in the last 45 years in the search for different types of drugs,[3,15,24–27,39–41] derivatives with potential use as antitumor agents from this class of pharmacological agents have not been reported previously. Still, several non-CA inhibitor sulfonamide derivatives were recently investigated for their anticancer properties. Thus, some arylsulfonyl-ureas/hydroxyguanidines or sulfonimideamides of types **9–11** have been reported by researchers from Eli Lilly[42] and by Chern *et al.*,[43] whereas Medina' s group at Tularik[44] prepared N-substituted polyhalogenobenzenesulfonamides of type **12** which strongly inhibited the growth of multidrug resistant MCF-7/ADR cancer cells in vitro. We stress again that sulfonamides **9–12** *do not* act as CA inhibitors, being substituted at the sulfonamido moiety with bulky groups which impair their binding to the Zn(II) ion of the enzyme and thus its inhibition.[45] It is assumed that the cytotoxicity of derivatives **9** might be a consequence of the uncoupling of mitochondria,[46] (in these organelle high concentrations of CA V are present, as stated earlier).[5] Still, up to now, no studies have been reported regarding the possibility that such anticancer compounds (of type **9–12**) might interfere with the CA activity, although in previous work from this laboratory,[47] we hypothesized as very probable a strong *in vivo* CA V inhibition (due to the hydrolysis of the cytotoxic agent, leading to the formation of unsubstituted sulfonamides as principal products).[42,48] On the other hand, it has been proven that the polyhalogeno-benzenesulfonamides **12** exert their cytotoxic activity due to irreversible binding to an amino acid residue

TABLE I CA inhibition data with standard inhibitors **1–3**, and the sulfonamides **4–8** against isozymes I, II and IV

Inhibitor	K_I* (nM)		
	hCA I[a]	*hCA II*[a]	*bCA IV*[b]
Acetazolamide, **1**	900	12	220
Methazolamide, **2**	780	14	245
Ethoxzolamide, **3**	25	8	13
4	8	7	30
5	10	6	25
6	12	20	30
7	49	10	21
8	36	8	23

* Standard error for the determination of K_I values was 10–15% (from 3 different assays); [a]Human (cloned) isozyme; [b] Isolated from bovine lung microsomes.

(Cys 239) of β-tubulin, by means of an aromatic nucleophilic substitution reaction involving the fluorine atom in position 4 as leaving group, resulting thus in the disruption of cellular microtubules.[44]

Inhibition data against three physiologically relevant CA isozymes, hCA I, hCA II and bCA IV with the derivatives **4–8** investigated here (Table I) show that all these compounds act as powerful CA I, CA II and CA IV inhibitors. All these sulfonamides inhibit in the nanomolar range isozymes CA II and CA IV and they were slightly less active CA I inhibitors. A notable exception from this behavior is given by the urea derivatives **4, 5** and **6** which act as very powerful CA I inhibitors too, with affinities of the same order of magnitude (in the nanomolar range) for CA I and CA II, and (slightly lower for) CA IV. A typical sulfonamide CA inhibitor (such as acetazolamide **1** or methazolamide **2**) generally shows a 50–75 times lower affinity for CA I as compared to CA II (ethoxzolamide **3** is an exception to this rule), and many aromatic/heterocyclic derivatives show the same type of activity.[3,15,24–30] In fact, in a previous communication[47] we showed that this type of urea derivatives might lead to the development of isozyme I-specific CA inhibitors, a goal difficultly accessible considered the high avidity for sulfonamides of isozymes CA II and CA IV.

The antitumor activity of the sulfonamide CA inhibitors **4–8** against a variety of cancer types/cell lines has been determined at the NIH National Cancer Institute, Bethesda, MD, USA, with samples furnished from this laboratory. A large variety of cancer/cell line types were included in these assays, such as leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer and prostate and breast cancer among others (Table II).

The following should be noted regarding the tumor cell growth inhibition data with the test compounds **4–8**: (i) different cancer cell lines, of the same tumor type, possessed a very variable response to inhibition of growth in the presence of the sulfonamide derivatives **4–8**. For example, the SR leukemia cells were very susceptible to inhibition by **6** (GI_{50} less than 10 nM), whereas other leukemia cell lines (such as MOLT-4 RPMI-8226 etc) showed the same level of inhibition only at concentrations between 3–16 μM of inhibitor. The same situation has been seen in the case of diverse breast cancer cell lines, with **6** acting as a very potent inhibitor ($GI_{50} = 50$ nM) against the MDA-MD-231/ATCC line, whereas the other breast cancer cell lines showed GI_{50} values in the range of 10–20 μM, (ii) all the investigated cancer lines were generally susceptible to inhibition by several of the sulfonamide tested (such as **5, 6** and **8**), but some cell lines, such as leukemia SR and CCRF-CEM, non-small cell lung cancer HOP-92, CNS cancer U251

TABLE II *In vitro* tumor cell growth inhibition data for the sulfonamide CA inhibitors **4–8**, against different tumors/cell lines

Tumor	*cell line*	GI_{50} (μM)*				
		4	**5**	**6**	**7**	**8**
Leukemia	K-562	> 100	26	3	> 100	20
	MOLT-4	> 100	34	3.5	> 100	12
	RPMI-8226	> 100	25	16	> 100	15
	SR	–	–	< 0.01	> 100	18
	CCRF-CEM	> 100	2.7	14	–	2
	HL-60(TB)	–	50	12	> 100	25
Non-small cell lung cancer	A549/ATCC	> 100	> 100	12.5	> 100	32
	HOP-92	37	51	19	4	1.7
	NCI-H460	> 100	65	8	> 100	35
	NCI-H322M	> 100	> 100	16	> 100	26.5
	NCI-H522	> 100	30	14	> 100	13
	EKVX	> 100	> 100	–	> 100	19
	HOP-62	> 100	53	16	–	–
	NCI-H226	> 100	> 100	–	96	19
	NCI-H23	> 100	> 100	12	> 100	20
Colon cancer	COLO-205	> 100	> 100	14.5	40	23
	HCT-15	> 100	> 100	7	> 100	20
	HT29	> 100	57	9	> 100	19.5
	SW-620	> 100	> 100	16	> 100	30
	HCT-116	> 100	82	12	> 100	39
	KM12	> 100	60	7	> 100	20
CNS cancer	SF-268	0.65	64	13	> 100	42
	SNB-19	> 100	> 100	12	> 100	40
	SNB-75	> 100	–	11.5	> 100	–
	U251	> 100	> 100	11	> 100	24
	SF-295	> 100	35	–	> 100	30
	SF-539	–	–	19	–	–
Melanoma	LOX IMVI	> 100	34	3	> 100	12.5
	MALME-3M	> 100	27	14	> 100	–
	M14	> 100	41	5	> 100	15
	SK-MEL-2	> 100	48	13	> 100	22.5
	SK-MEL-28	> 100	> 100	18	> 100	34
	SK-MEL-5	> 100	68	16	> 100	14
	UACC-62	> 100	63	85	> 100	26
	UACC-257	–	77	18	> 100	10
Ovarian cancer	IGROV1	> 100	59	17	> 100	8
	OVCAR-4	> 100	45	13	> 100	27
	OVCAR-5	> 100	> 100	10	> 100	42
	OVCAR-8	> 100	> 100	15	> 100	13
	OVCAR-3	> 100	19	–	> 100	18.5
	SK-OV-3	> 100	–	17	> 100	24
Renal cancer	768–0	> 100	69	13.5	> 100	30
	ACHN	> 100	> 100	8	> 100	23
	CAKI-1	> 100	67	–	> 100	21
	RXF 393	> 100	22	14	> 100	–
	UO-31	> 100	> 100	10	> 100	20
	SN12C	> 100	> 100	14	> 100	19.5
	TK-10	–	50	23	> 100	32.5
	A498	–	–	1	–	–

TABLE II (*continued*)

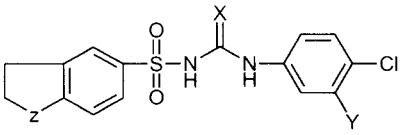
Tumor	*cell line*	GI_{50} (μM)*				
		4	**5**	**6**	**7**	**8**
Prostate cancer	PC-3	> 100	51	10	> 100	24
	DU-145	> 100	43	14.5	> 100	–
Breast cancer	MCF7	> 100	52	15	> 100	35
	MDA-MB-435	> 100	> 100	–	> 100	67
	MDA-N	> 100	32	13	> 100	25
	BT-549	> 100	87	11	> 100	16
	T-47D	> 100	48	10	–	–
	NCI/ADR-RES	> 100	> 100	11.5	> 100	19
	HS 578T	–	> 100	19.5	> 100	17
	MDA-MB-231/ATCC	> 100	> 100	0.05	> 100	33

* Molarity of inhibitor producing a 50% inhibition of growth of the tumor cells after 48 h exposure to variable concentrations (10^{-4}–10^{-8} M) of the test compound. Errors were in the range of ±5–10% (from two determinations).

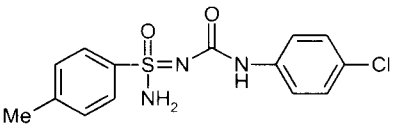
and melanoma LOX IMVI, responded quite well to this type of cell growth inhibitor, (iii) important differences in activity between the investigated sulfonamides were detected. Thus, two compounds, namely **4** and **7**, were generally much less effective tumor cell growth inhibitors compared to the other investigated compounds, **5, 6** and **8**. The first sulfonamide, **4**, inhibited significantly only two cell lines (HOP-92 and SF-268). Its diphenyl-substituted congener **5**, showed a dramatic (and positive) enhancement of activity, since this derivative inhibited the largest majority of the investigated tumor cell lines with GI_{50} values in the range of 2.7–87 μM. The most active derivative against the largest majority of the investigated tumor cell lines (except a small number of lines, such as CCRF-CEM, HOP-92, SF-268, SK-MEL-5UACC-62 and UACC-257) was **6**. The benzenedisulfonamide **7**, possessing the 3,4-dichloroureido moiety also present in **6**, was on the other hand much less active with a behavior more similar to that of **4**. The diphenylcarbamoyl derivative **8** was again much more active than the structurally related derivative **7**, showing a behavior similar to that of **6**. One must note that the most lipophilic compounds in the investigated series (**5, 6** and **8**) were the best tumor cell growth inhibitors, (iv) the inhibition of growth of tumor cells was dose-dependent on the concentration of sulfonamide inhibitor used in the experiments (data not shown), with growth inhibition increasing at increasing sulfonamide concentrations.

It should also be noted that the sulfonamides investigated here seem to be much more potent tumor cell growth inhibitors as compared to methazolamide **2** or ethoxzolamide **3**, previously studied by Chegwidden and

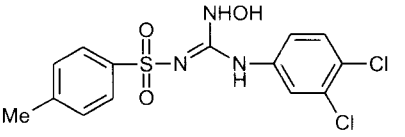
Spencer,[21b] although the cell lines and methods used to assay the inhibition of growth in this and the above-cited[21b] study are quite different.



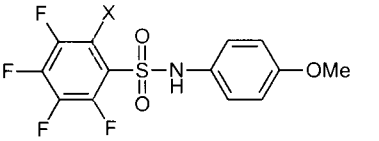
9a: X = O, Y = H, Z = CH_2 - sulofenur
9b: X = O, Y = Cl, Z = O - LY295501
9c: X = S, Y = H, Z = CH_2



10



11



12: X = H, F, Br

The mechanism of tumor growth inhibition with these sulfonamides is not known for the moment, but several hypothesis may be proposed in this regard. Thus, as suggested by Chegwidden and Spencer,[21b] these compounds, similarly to the classical inhibitors methazolamide **2** or ethoxzolamide **3**, might reduce the provision of bicarbonate needed for the nucleotide synthesis mediated by carbamoyl phosphate synthetase II. An alternative/additional mechanism might involve the acidification of the intracellular milieu as a consequence of CA inhibition by these potent CA inhibitors, as shown in the seminal work of Teicher *et al.*[20] on the enhanced anticancer activity of different drugs in combination with acetazolamide **1**. It is also possible that the sulfonamides reported here interfere with the activity of the CA isozymes present predominantly in tumor cells, such as CA IX,[8] XII[11] and XIV,[12] but this hypothesis is difficult to verify at the present time, since clones of these new isozymes are not readily available. Finally, a mechanism based on uncoupling of mitochondria,[46,47] as for the relatively similar diarylsulfonylureas **9, 10** mentioned above should not be excluded, especially taking into account the fact mentioned above that the more lipophilic derivatives (**5**, **6** and **8**) acted as better tumor cell growth inhibitors compared to their more hydrophilic counterparts (**4** and **7**). It is assumed that a lipophilic derivative should more easily cross lipid membranes (cell membrane as well as mitochondrial membranes) in order to arrive at the site of action (the mitochondria in this specific case) as compared to a highly polarized (more hydrophilic) derivative.[26,27] It should be noted that a

natural concentrating mechanism for such a process might function since the mitochondria contain high amounts of CA V, an isozyme with affinity of the same order of magnitude for sulfonamides as CA IV investigated in the present study.[49] This is for the moment only a hypothesis that needs to be verified.

In conclusion, we report here that some benzenesulfonamide/disulfonamide derivatives possessing strong CA inhibitory properties also act as powerful tumor cell growth inhibitors *in vitro*, with GI_{50} values in the 10 nM–30 · nM range. Some hypothesis regarding the mechanism of antitumor activity of these derivatives have been presented.

Note

Since this manuscript has been submitted, Sly's group[50] reported acetazolamide **1**, to act as a potent inhibitor on the invasive capacity of four renal carcinoma cell lines (Caki-1, Caki-2, ACHN, and A-498), some of which have also been investigated by us in the present paper. Thus, the promising anticancer properties of sulfonamide CA inhibitors might indeed lead to interesting therapeutic applications.

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